Structural Studies of The Human Particulate Guanylyl Cyclase Receptor A (Pgc-A), To Support Therapeutics for Cardiovascular Diseases.

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This study addresses a huge need for innovative and effective therapies to reduce hypertension (1). Cardiovascular diseases (CVD) are the leading cause of death worldwide (1). In the United States, health care costs for heart failure are expected to double by the next decade (1). A major contributor to these disorders is hypertension. The available remedies for treatment of hypertension have side effects, and some individuals show resistance to treatments. Resistant hypertension will lead to heart failure, stroke, and metabolic syndromes and currently no medications are available to treat resistant hypertension (2). A more comprehensive understanding of the regulation of blood pressure is required to develop effective drugs against hypertension. One of the regulators is particulate guanylyl cyclase-A (pGC-A), which is a membrane receptor protein that is highly expressed in the heart, kidney, adrenals, vasculature, and adipocytes (4). The heart is also an endocrine organ that generates the hormone atrial natriuretic peptide (ANP), which binds to and activates pGC-A and thereby plays a key role in maintaining healthy blood pressure levels and metabolic balance. However, ANP is also rapidly degraded by the membrane-bound enzyme neprilysin (4), limiting its biological activity. Generally, drug molecules block enzymes or receptors in the treatments against CVD, but in this approach, the pGC-A receptor will be activated by ANP-like drugs, prompting metabolic changes that lower blood pressure, decrease glucose levels, and diminish the formation of arterial plaque (4). In this work, we collaborate with Dr. John C. Burnett Jr., M.D., from the Mayo Clinic, who has developed a modified form of ANP, called MANP that is currently in Phase 1 clinical trials and has shown protection from hypertension, obesity, and other metabolic imbalances (5). pGC-A contains extracellular, transmembrane, and intracellular domains. The intracellular domain is divided into the protein kinase like domain and the guanylyl cyclase domain (GCD). The extracellular domain (ECD) of pGC-A interacts with ANP, which triggers the intracellular GCD to convert guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). cGMP is a second messenger that interacts with a variety of effector molecules downstream to evoke different cellular effects in the cardiovascular system (6). The studies conducted in our lab found that the functional form of pGC-A could be a tetramer. Also, previous studies by our group found (7) that ATP elevates the catalytic activity but is not crucial for the activation of this receptor. Also, it was found that the monomers in the tetramer were oligomerized not through disulfide bonds. A three-step mechanism was proposed for the pGC-A activation from these findings (7). The pGC-A tetramer contains two functional units, and each unit contains a homodimer and is tetramerized by non-covalent interactions. The first step is binding of ANP that initially activates the pGC-A, the second step is the binding of ATP that further elevates the pGC-A activity, and the third step is the phosphorylation of pGC-A that fully activates this receptor (7). pGC-A is a membrane protein, a class of proteins for which structure determination is challenging because of their amphiphilic nature (8). Currently, the only partial structures of pGC-A are published for the ECD from rat (9), thus no structure has been determined for the full-length receptor. To understand the molecular mechanism underlying the ANP/pGC- A/cGMP signaling pathway and to effectively regulate pGC-A, structural data on the whole receptor protein including its transmembrane and intracellular domains is required. The research seeks to improve the conception of physiological processes that are stimulated by pGC-A activation and decipher the mechanism of how ANPs fight against CVD by analyzing the ligand receptor interactions in terms of structure and dynamics. The three-dimensional atomic resolution structure of the membrane-translocated full-length pGC-A with and without the ANP would be achieved by advanced structural biology techniques such as X- ray crystallography and cryogenic electron microscopy (cryo-EM). Currently, the pGC-A is successfully expressed, purified, and preliminary serial crystallography diffraction patterns were obtained. The structure determination of the pGC-A complex with native ANP as well as with MANP would be an important discovery in the field that would aid design of next generation MANP analogues in the fight against CVDs.

Refernces

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